22. (Amended) The method of claim 21 wherein each of said electrophoretic probes comprises a target-binding moiety and wherein the target-binding moiety and said second reagent are each antibodies or fragments thereof.

23. (Amended) The method according to claim 19, 20, 21, or 22 wherein said eTag reporter are identified by fluorescence or by an electrochemical label, and wherein said step of providing one or more electrophoretic probes includes providing a plurality of from 5 to 100 said electrophoretic probes.

REMARKS

Claims 5, 6, 8-11, 13-14, 16, 19-20, and 22-23 have been amended and claims 8, 9 and 24 have been cancelled. Claims 5-7, 10-23, and 25 are currently pending in the application. All pending claims are set forth in Exhibit A with amendments shown (if applicable).

Applicants gratefully acknowledge the 14 February 2003 interview with the Examiner in which all pending rejections in the application were discussed. During the interview, the Examiner agreed that the present amendments have addressed the concerns raised over new matter.

Basis for the amendments are as follows:

Claim(s)	Term/Phrase	Basis
5	"plurality" in reference to the number of electrophoretic probes in an assay.	Page 9, lines 43-45.
5-6, 10-11, 13-14, 16, 22, 23	"electrophoretic probe"	Page 9, lines 24-32. Claim 1.
5, 10, 22	"target-binding moiety" in reference to a component of an electrophoretic probe.	Page 9, lines 24-32.
5, 19	"electrophoretic mobility" in reference to eTag reporters.	Page 4, lines 30-32 and 35-38.
5	"that are cleaved based on oxidation" in reference to cleavable linkages.	Page 19, line 10, of parent application 09/698,846*
14, 19, 22	"second reagent"	Page 18, line 22, of parent application 09/698,846*
10	"comprising a detection group, D, and a mobility modifier, M," in reference to released eTag reporters.	Page 4, lines 15-23.
22	"antibodies or fragments thereof" in reference to target-binding moiety or second reagent.	Table 4, page 33. Page 13, lines 35-43. Claim 38 of parent application 09/698,846 that was incorporated by reference.

* Parent application 09/698,846 was incorporated by reference (see page 1, lines 5-8, of the specification), and the indicated passages were expressly incorporated by the 22 July 2002 Amendment.

No new matter has been added by the amendments. Reconsideration is respectfully requested.

Rejections Under 35 U.S.C. 112 First Paragraph

In paragraph 4 of the Office Action, the Examiner rejected claims 5-25 under 35 U.S.C. 112 first paragraph because of the use of various terms allegedly lacking support in the specification introduced new matter.

In view of the amendments, Applicants submit that the concerns raised by the Examiner over new matter have been addressed and respectfully request that the above rejection be withdrawn.

Rejections Under 35 U.S.C. 112 Second Paragraph

In paragraph 6 of the Office Action, the Examiner rejected claims 10-13 under 35 U.S.C. 112 second paragraph. The Examiner avers that the clause "wherein upon cleavage M and D impart on said eTag reporter a distinct mass/charge ratio" is unclear because it does not indicate whether cleavage occurs between the detection group, D, and the antibody binding compound or between the detection group, D, and the mobility modifier, M.

Applicants respectfully disagree with this rejection, particularly in view of the amendments. The amendment to claim 10 adds the phrase "comprising a detection group, D, and a mobility modifier, M," that make it clear and unambiguous that a released eTag reporter has a detection group AND a mobility modifier moiety. Consequently, no cleavage occurs between the detection group and the mobility modifier of an eTag reporter in Applicants' method.

Accordingly, Applicants respectfully request that the rejection be withdrawn.

Rejection Under 35 U.S.C. 102

In paragraph 8 of the Office Action, the Examiner rejected claims 5-6, 9 and 18 under 35 U.S.C. 102(b) as being anticipated by Bocuslaski et al (U.S. patent 4,331,590). The Examiner argues as follows: Bocuslaski discloses an indicator moiety attached to a ligand, such as an antibody. The indicator moiety consists of a dye attached to the ligand by one linkage and attached to a glycone moiety by another linkage that is enzymatically cleavable, such that when the linkage is cleaved the glycone moiety is released the dye becomes fluorometrically or colorimetrically active. Bocuslaski further discloses a separation step, thereby anticipating the embodiment of

Applicants' invention wherein a single target compound is detected by a single electrophoretic probe.

Applicants respectfully disagree, particularly in view of the amendments. In order to expedite the prosecution, Applicants have amended without prejudice the above claims so that a plurality of electrophoretic probes is employed in Applicants' method, thereby obviating the concern raised by the Examiner with respect to 35 U.S.C. 102. Nonetheless, even without the amendment, Applicants submit that the unamended claims are not anticipated by Bocuslaski. In particular, the separation step disclosed by Boscuslaski is merely a wash step in a heterogeneous assay format to remove unbound ligand-indicator moiety conjugates. It is not a separation of the released glycone moieties. Boscuslaski discloses that the detectable part of the indicator moiety is the dye that remains attached to the ligand. There is no disclosure or suggestion in Bocuslaski that the detectable portion of the indicator moiety is separated from the assay reaction for detection, as called for in Applicants' invention.

In view of the above, Applicants respectfully request that the rejection be withdrawn.

Rejections Under 35 U.S.C. 103

In paragraph 10 of the Office Action, the Examiner rejected claims 7-8 under 35 U.S.C. 103(a) as being obvious over Bocuslaski et al (U.S. patent 4,331,590) as applied to claims 5-6 and 18 above, and further in view of Kameda et al (U.S. patent 4,780,421). Bocuslaski is applied as above and Kameda discloses the use of cleavable linkages in specific binding assays, e.g. immunoassays, to permit the release of a labeling moiety, e.g. a fluorescent label, from a binding complex in order to obtain an improved signal. The Examiner argues that one of ordinary skill in the art would have been motivated to use the cleavable linkages of Kameda (which include linkages cleavable by oxidation) in the assay of Bocuslaski because they lead to increased sensitivity and are easy to apply.

Applicants respectfully disagree, particularly in view of the amendments. There are several fundamental differences between Bocuslaski and Applicants' invention that make it nonobvious over Bocuslaski to one of ordinary skill in the art and these differences are not lessened by the disclosure of Kameda. First, Applicants' invention is presently directed to the use of *a plurality* of electrophoretic probes in an assay to detect a plurality of analytes, whereas both Bocuslaski and Kameda are directed to the detection of only a single analyte in an assay. Bocuslaski's assay is incapable of such multiplexed measurements because it relies solely on the change in light-generating properties of optical indicator molecules to signal the presence or

absence of an analyte. In both Kameda and Bocuslaski, upon cleavage there is an increase in the signal generated by a label, usually a fluorescent label. Both references also teach that only a single kind of label is used at a time in a given assay. Thus, if more than one kind of binding compound were used in the same assay, one could not determine which target analyte was being detected because the same signal would be generated for both. Neither Kameda nor Bocuslaski disclose or suggest any means for distinguishing more than one label in the same reaction, and neither reference discloses or suggests the advantages or desirability of making such multiplexed measurements.

Second, in Applicants' invention, cleavage results in the *release of an eTag reporter from a binding compound* where the eTag reporter is then separated and detected. In Bocuslaski, on the other hand, cleavage increases the intensity of a signal from an indicator molecule that remains attached to a binding compound. *There is no release of the indicator from the binding compound.* Kameda teaches the release of a fluorescent label from a binding complex, but only to overcome self-quenching caused by the close proximity of fluorecent labels to one another in a binding complex (col. 6, lines 14-17). Kameda does not disclose or suggest the separation of the released labels from the assay mixture, as called for in Applicants' method.

Third, as just noted, Applicants' invention calls for separation of the cleaved eTag reporters, whereas there is no separation of cleaved indicator moieties in Bocuslaski's assay or cleaved labels in Kameda's assays. The separation step disclosed in Bocuslaski (col. 10, lines 60-67) is merely to remove unbound indicator-binding compound conjugates from specifically bound conjugates prior to cleavage of indicator moieties in a heterogeneous assay format. Bocuslaski neither discloses nor suggests the separation of cleaved indicator molecules after cleavage which, of course, makes sense for the single-plex as says disclosed. The same is true for Kameda.

Fourth, the optical properties of Applicants' *eTag reporters do not change after cleavage*, whereas in both Kameda and Bocuslaski the object of the invention is to permit an increase in signal after a cleavage reaction. It is not necessary for the optical properties of the eTag reporters to change after cleavage because they are separated from the assay reaction and from one another for detection. This is in contrast to both Kameda and Bocuslaski where only single-plex assays are contemplated, so separation is not necessary and the presence of a single analyte is indicated solely by changes in the optical properties of a cleaved indicator.

In summary, Applicants submit that one of ordinary skill would not be motivated to derive Applicants' invention from either Kameda's or Bocuslaski's disclosures, either alone or in combination, because (i) they provides no teaching or suggestion of the advantages, desirability, or

technical challenges of detecting multiple analytes in a single assay, (ii) the disclosed assays are inherently suitable only for detecting a single analyte because only a single optical signal is generated, (iii) Both Kameda's and Bocuslaski's assay schemes operate on different principles than that of Applicants' invention (Bocuslaski: cleavage to change *indicator that stays attached to binding component*; Kameda: cleavage to release dye to overcome self-quenching; Applicants: cleavage to *release a plurality of eTag reporters from one or more binding complexes followed by separation and detection*), and (iv) even if the assays could be adapted to detect multiple analytes, neither Bocuslaski nor Kameda, either alone or in combination, provide any hints or suggestions of how this might be carried out.

In view of the above, Applicants submit that their invention would not be obvious to one of ordinary skill over the disclosures of Bocuslaski and Kameda. Accordingly, Applicants respectfully request that the above rejection be withdrawn.

In paragraph 11 of the Office Action, the Examiner rejected claims 10-17 and 19-24 under 35 U.S.C. 103(a) as being obvious over Bocuslaski et al (U.S. patent 4,331,590) as applied to claims 5-6 and 18 above, and further in view of Giese (Analytical Chemistry, 2: 166-168 (1983)) and Breslow et al (U.S. patent 6,331,530). The Examiner argues as follows: Bocuslaski discloses ligands attached to indicator moieties each containing an enzymatically cleavable linkage, such that when the cleavable linkage is broken the indicator moiety becomes optically distinguishable from indicator moieties having intact linkages. Giese discloses sets of releasable electrophoric tags that may be attached to binding compounds and used in binding assays to detected multiple analytes in a single reaction. Released electrophoric tags are identified after separation by gas chromatography with electron capture detection. Breslow discloses a compound for cancer therapy consisting of cyclodextrin dimers whose cyclodextrin pairs are connected by a linkage which is cleaved by singlet oxygen generated by a photosensitizer. The purpose of Breslow's compound is to create a locally elevated concentration of photosensitizers in a region (e.g. by a tumor) of illumination. From Giese's teaching of the potential usefulness of multiplexed assays, one of ordinary skill in the art would have been motivated to combine the assays of Bocuslaski with the multiplexing and releasable tag of Giese to obtain Applicants' invention. Brelow teaches the use of a sensitizer to generate singlet oxygen that cleaves a chemical bond thereby releasing cyclodextrin monomers. Thus, because of its efficiency in cancer therapy, one of ordinary skill in the art would be motivated to incorporate the sensitizer-based cleavage system of Breslow into the assay of Giese and Bocuslaski.

Applicants respectfully disagree, particularly in view of the amendments. Applicants' invention employs *electrophoretic separation* of eTag reporters whereas Giese employs *gas chromatographic separation* of electrophoric "release" tags. This is an important difference as the gas phase separation of Giese limits the nature of the released tags that can be used and requires that additional steps be performed prior to separation, and neither Bocuslaski nor Breslow disclose or suggest how to overcome this shortcoming.

In regard to the additional steps, in the suggested binding assays, Giese's method requires that "release" tags be extracted from the assay mixture into a volatile organic solvent, then concentrated by evaporation prior to injection into a gas chromatograph (page 167, col. 2, last sentence in first full paragraph). This is a time consuming and labor intensive step that is not required by Applicants' method. In accordance with Applicants' method, *released eTag reporters can be separated by electrophoresis directly from an assay reaction mixture.*

In regard to the nature of tags that can be separated by gas chromatography, Applicants direct the Examiner's attention to the following excerpt from a description of gas chromatography on the Agilent, Inc. website:

> "It is estimated that 10-20% of the known compounds can be analyzed by GC. To be suitable for GC analysis, a compound must have sufficient volatility and thermal stability. If all or some of a compound's molecules are in the gas or vapor phase at 400-450°C or below, *and they do not decompose at these temperatures*, the compound can probably be analyzed by GC."(www.chem.agilent.com/cag/cabu/whatisgc.htm) (copy attached as Exhibit B). (Emphasis added).

Many optically active molecules, such as organic fluorescent molecules, are not available for use in Giese's method because such molecules decompose rather than volatilize at temperatures necessary for gas chromatography (e.g. rhodamine B decomposes at 211°C, carboxyfluorescein decomposes at 275°C, and the like, data from Aldrich catalog). This is not a limitation in Applicants' invention because electrophoretic separation takes place in aqueous conditions that (by definition) must be less than boiling temperature, i.e. less than 100°C.

Applicants submit that neither Bocuslaski nor Giese nor Breslow disclose or suggest alone or in combination the electrophoretic separation of molecular tags, such as eTag reporters. In fact, Applicants submit that Giese teaches away from the combination with Bocuslaski because the latter reference teaches the use of fluorescent organic molecules as labels—a class of labels that are generally not amenable to gas chromatographic analysis. (Applicants note that conventional detectors in gas chromatographs are based on thermal conductivity, flame ionization, electron

capture, or mass spectrometry, e.g. Harris, "Exploring Chemical Analysis," 2nd edition (Freeman, San Francisco, 2001), none of which are suitable for organic fluorescent molecules.) Therefore, application of the multiplexing taught by Giese, which depends on gas chromatography, would not have been obvious to one of ordinary skill practicing the single-plex fluorescent labels of Bocuslaski, regardless of whether or not the labels are cleaved using the sensitizer-based system of Breslow.

In regard to claims 14-18 and 19-25, Applicants further point out that none of cited references disclose or suggest a *second reagent* that is capable of generating an active species for cleaving the cleavable linkage. Applicants submit that one of ordinary skill in the art having knowledge of Bocuslaski, Giese, and Breslow clearly would not have been led to these embodiments of the invention without making an independent inventive contribution.

For the above reasons, Applicants submit that the above rejection is inappropriate and respectfully request that it be withdrawn.

In view of the above, Applicants submit that the claims as written fully satisfy the requirements of Title 35 of the U.S. Code, and respectfully request that the rejections thereunder be withdrawn and that the claims be allowed and the application quickly passed to issue.

If any additional time extensions are required, such time extensions are hereby requested. If any additional fees not submitted with this response are required, please take such fees from deposit account **50-2266**.

Respectfully submitted,

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Exhibit A
Claims showing Insertions and Deletions

5. (Amended) A method for determining the presence or absence of one or more target compounds in a sample, the method comprising the steps of:

providing [~~one or more~~] a plurality of electrophoretic probes [~~antibody binding compounds~~] specific for the one or more target compounds [~~a target compound~~], each electrophoretic probe [~~antibody binding compound~~] having a target-binding moiety and one or more eTag reporters attached by cleavable linkages that are cleaved by oxidation, the one or more eTag reporters of each electrophoretic probe [~~antibody binding compound~~] being distinguished from those of other electrophoretic probes [~~antibody binding compounds~~] by electrophoretic mobility [~~one or more physical characteristics~~];

combining with the sample [~~one or more~~] the plurality of electrophoretic probes [~~antibody binding compounds for each of the target compounds~~] such that in the presence of a target compound a complex is formed between each target compound and [~~the~~] one or more electrophoretic probes [~~antibody binding compounds~~] specific therefor;

cleaving the cleavable linkages of each electrophoretic probe [~~antibody binding compound~~] forming such complex so that eTag reporters are released; and

separating and identifying the released eTag reporters based on electrophoretic mobility [~~the one or more physical characteristics~~] to determine the presence or absence of the one or more target compounds.

6. (Amended) The method of claim 5 further including a step prior to said step of cleaving, the step comprising separating said complexes from unbound said electrophoretic probes [~~antibody binding compounds~~].

7. The method of claim 6 wherein said cleavable linkages are each an olefin, a thioether, a sulfoxide, or a selenium analog of the thioether or sulfoxide.

8. (Cancelled) [~~The method of claim 7 wherein said step of cleaving includes oxidizing said cleavable linkages to release said eTag reporters.~~]

9. (Cancelled) [~~The method according to claim 5, 6, 7, or 8 wherein each of said eTag reporters has a fluorescent label or an electrochemical label, and wherein said one or more physical characteristics are electrophoretic mobility or fluorescence~~].

10. (Amended) The method according to claim 5, 6, or 7 [~~of claim 9~~] wherein each of said electrophoretic probes [~~antibody binding compound~~] is selected from a group defined by the formula:

$$[(M, D)\text{-}L]_k\text{-}T$$

wherein:

T is a target-binding moiety [~~an antibody~~] specific for one of said one or more target compounds [~~said target compound~~];

k is an integer in the range of from 1 to 10;

L is said cleavable linkage that is cleaved by oxidation;

D is a detection group; and

M is a mobility modifier consisting of from 1 to 500 atoms selected from the group consisting of carbon, hydrogen, oxygen, nitrogen, sulfur, phosphorus, and boron; and wherein upon cleavage [~~M and D impart on~~] said eTag reporter comprising a detection group, D, and a mobility modifier, M, has a distinct mass/charge ratio so that said eTag reporters from different electrophoretic probes [~~antibody binding compounds~~] form distinct peaks upon electrophoretic separation.

11. (Amended) The method of claim 10 wherein said mass/charge ratio is in the range of -0.001 and 0.5, and wherein said step of providing includes providing a plurality of from 5 to 100 said electrophoretic probes [~~antibody binding compounds~~].

12. The method of claim 11 wherein k is in the range of from 1 to 3, and wherein M is a mobility modifier consisting of from 1 to 300 atoms selected from the group consisting of carbon, hydrogen, oxygen, nitrogen, sulfur, phosphorus, and boron.

13. (Amended) The method of claim 12 wherein said plurality of said electrophoretic probes [~~antibody binding compounds~~] is in the range of from 5 to 50.

14. (Amended) The method of claim 5 wherein [~~said cleavable linkage is cleaved by oxidation, wherein said one or more physical characteristics are electrophoretic mobility or fluorescence, and wherein~~] said step of cleaving includes providing a second reagent [~~antibody binding compound~~] specific for each of said one or more target compounds, each second reagent being capable of [~~antibody compound having a sensitizer for~~] generating an active species for oxidizing said cleavable linkage.

15. The method of claim 14 wherein said active species is singlet oxygen and said cleavable linkage is an olefin, a thioether, a sulfoxide, or a selenium analog of the thioether or sulfoxide.

16. (Amended) The method according to claim 14 or 15 wherein said step of providing includes providing a plurality of from 5 to 100 said electrophoretic probes [~~antibody binding compounds~~].

17. The method of claim 16 wherein said mobility modifier consisting of from 1 to 300 atoms selected from the group consisting of carbon, hydrogen, oxygen, nitrogen, sulfur, phosphorus, and boron.

18. The method of claim 17 wherein said detection group comprises a fluorescent label or an electrochemical label.

19. (Amended) A method for determining the presence or absence of one or more target compounds in a sample, the method comprising the steps of:

providing one or more electrophoretic probes [~~binding compounds~~] specific for each of the one or more target compounds, each electrophoretic probe [~~binding compound~~] having one or more eTag reporters attached thereto by a cleavable linkage, the one or more eTag reporters of each electrophoretic probe [~~binding compound~~] being distinguished from those of other binding compounds by electrophoretic mobility [~~one or more physical characteristics~~];

providing a second reagent [~~binding compound~~] specific for each of the one or more target compounds, each second reagent being capable of [~~binding compound having a sensitizer for~~] generating an active species;

combining with the sample one or more electrophoretic probes [~~binding compounds~~] and a second reagent [~~binding compound~~] for each of the one or more target compounds such that in the presence of a target compounds a complex is formed between the target compound, the one or more electrophoretic probes [~~binding compounds~~] specific therefor, and the second reagent [~~binding compound~~] specific therefor, and such that [~~the sensitizer of~~] the second reagent [~~binding compound~~] causes the generation of an active species and the cleavage of one or more cleavable linkages to release one or more eTag reporters; and

electrophoretically separating and identifying the one or more released eTag reporters to determine the presence or absence of the one or more target compounds.

20. (Amended) The method of claim 19 wherein said cleavable linkage is cleaved by oxidation [~~and wherein said active species is singlet oxygen or hydrogen peroxide~~].

21. The method of claim 20 wherein said active species is singlet oxygen and said cleavable linkage is an olefin, a thioether, a sulfoxide, or a selenium analog of the thioether or sulfoxide.

22. (Amended) The method of claim 21 wherein each of said electrophoretic probes comprises a target-binding moiety and wherein the target-binding moiety and said second reagent [~~said binding compound and said second binding compound~~] are each antibodies or fragments thereof [~~antibody binding compounds~~].

23. (Amended) The method according to claim 19, 20, 21, or 22 wherein said eTag reporter are identified by fluorescence or by an electrochemical label, and wherein said step of providing one or more electrophoretic probes [~~binding compounds~~] includes providing a plurality of from 5 to 100 said electrophoretic probes [~~binding compounds~~].

24. (Cancelled) [~~The method of claim 23 wherein said sensitizer generates singlet oxygen upon photoactivation.~~]

25. The method according to 19, 20, 21, or 22 further including, prior to said step of electrophoretically separating, a step of separating said released eTag reporters from any components that interfere with electrophoretic analysis.

Agilent - Life Sciences/Chemical Analysis Online Store

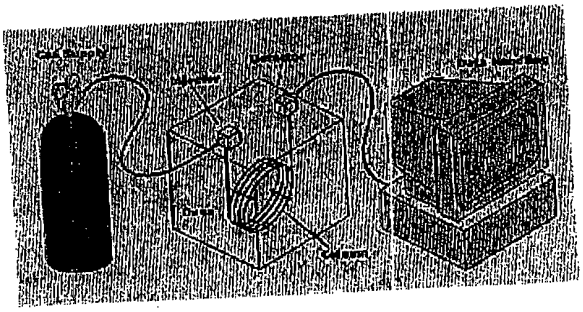
Exhibit B

What is Gas Chromatography?

Chromatography is the separation of a mixture of compounds (solutes) into separate components. By separating the sample into individual components, it is easier to identify (qualitate) and measure the amount (quantitate) of the various sample components. There are numerous chromatographic techniques and corresponding instruments. Gas chromatography (GC) is one of these techniques. It is estimated that 10-20% of the known compounds can be analyzed by GC. To be suitable for GC analysis, a compound must have sufficient volatility and thermal stability. If all or some of a compound's molecules are in the gas or vapor phase at 400-450°C or below, and they do not decompose at these temperatures, the compound can probably be analyzed by GC.

The main parts of a basic GC system are shown in Figure 1. One or more high purity gases are supplied to the GC. One of the gases (called the carrier gas) flows into the injector, through the column and then into the detector. A sample is introduced into the injector usually with a syringe or an exterior sampling device. The injector is usually heated to 150-250°C which causes the volatile sample solutes to vaporize. The vaporized solutes are transported into the column by the carrier gas. The column is maintained in a temperature controlled oven. The solutes travel through the column at a rate primarily determined by their physical properties, and the temperature and composition of the column. The various solutes travel through the column at different rates. The fastest moving solute exits (elutes) the column first then is followed by the remaining solutes in corresponding order. As each solute elutes from the column, it enters the heated detector. An electronic signal is generated upon interaction of the solute with the detector. The size of the signal is recorded by a data system and is plotted against elapsed time to produce a chromatogram.

Figure 1. The Basic Components of a GC System



The ideal chromatogram has closely spaced peaks with no overlap of the peaks. Any peaks that overlap are called coeluting. The time and size of a peak are important in that they are used to identify and measure the amount of the compound

in the sample. The size of the resulting peak corresponds to the amount of the compound in the sample. A larger peak is obtained as the concentration of the corresponding compound increases. If the column and all of operating conditions are kept the same, a given compound always travels through the column at the same rate. Thus, a compound can be identified by the time required for it to travel through the column (called the retention time). The identity of a compound cannot be determined solely by its retention time. A known amount of an authentic, pure sample of the compound has to be analyzed and its retention time and peak size determined. This value can be compared to the results from an unknown sample to determine whether the target compound is present (by comparing retention times) and its amount (by comparing peak sizes). If any of the peaks overlap, accurate measurement of these peaks is not possible. If two peaks have the same retention time, accurate identification is not possible. Thus, it is desirable to have no peak overlap or co-elution.

Inside a Capillary GC Column

capillary GC column is comprised of two major parts - tubing and stationary phase. A thin film (0.1-10.0 µm) of a high molecular weight, thermally stable polymer is coated onto the inner wall of small diameter (0.05-0.53 mm I.D.) tubing. This polymer coating is called the stationary phase. Gas flows through the tubing and is called the carrier gas or mobile phase.

Upon introduction into the column, solute molecules distribute between the stationary and mobile phases. The molecules in the mobile phase are carried down the column; the molecules in the stationary phase are temporarily immobile and do not move down the column. As the molecules in the mobile phase move through the column, some of them eventually collide with and re-enter the stationary phase. During the same time span, some of the solute molecules leave the stationary phase and enter the mobile phase. This occurs thousands of times for each solute molecule as it passes through the column. All of the molecules corresponding to a specific compound travel through the column at nearly the same rate and appear as a band of molecules (called the sample band). The goal is to have no overlap between adjacent sample bands as they exit the column. This is accomplished by making each sample band travel at a different rate and by minimizing the width of the sample band. The rate at which each sample band moves through the column depends on the structure of the compound, the chemical structure of the stationary phase and the column temperature. The width of the sample band depends on the operating conditions and the dimensions of the column. The proper column and operating conditions are critical in obtaining no, or the least amount of, peak co-elution.